NAC 4

REPORT ON RESEARCH CONTRACT #R 38 WITH NASA COVERING CONTRACT YEAR 1965

12/10/65

I. MOLECULAR ENERGETICS

(A) Microcalorimetry

The year of 1965 brought the general introduction of microcalorimetry into the laboratories of the chemical and biological sciences, with the instrument developed at the Bio-energetics Laboratories, and tested in work under contract #R 8 and #R 38. Serial construction of Heatburst or Pulse-microcalorimeters made possible not only their general use, but also substantial improvements of their performance. More closely matching thermopiles were obtained. Combination of these piles with optimally suited amplifying and recording equipment resulted in a three fold increase of the voltage-sensitivity. Automatic integration eliminated human errors in planimetry. Zero spikes (a limiting characteristic) were substantially reduced in size. Baseline stability was improved. A temperature-controlled enclosure will further increase the stability and will permit investigations over a wide range of temperatures. Meanwhile, at the Bio-energetics Laboratories new vessels were developed which have greatly improved the speed of response. These are important steps toward an even broader application of microcalorimetry as a tool of chemical analysis and chemical thermodynamics.

As a result of these developments the potential of microcalorimetry as a method for life-detection was explored in a study by Thomas B Weber (NASA Contract No. NAS 2-2554) titled "Detection of Extraterrestrial Microorganisms by Microcalorimetry". In this study the rates of metabolic (microbial) heat output ranged from 20 to 800 microcalories per second in soil-samples of 200 milligrams. The authors report: "Minimal detectable (heat) flux is set by the characteristic signal drift of the instrument, this being essentially dependent on ambient temperature-variations. The Benzinger instrument, in an environment having a maximum total diurnal temperature excursion of 4°C, shows a drift of 0.06 microvolts per hour, or (in heat flux equivalent 0.25 microcalories per second per hour ". "The present study indicates clearly that microcalorimetry provides a feasible, convenient and sensitive experiment for the detection of extraterrestrial life." It must be emphasized, however, that much remains to be done in reducing the basic potential of the method to practice. In the coming contract year the Bio-Energetics Laboratories will continue efforts under contract # R-38 to further improve the detection-sensitivity and to reduce the size and weight of instrumentation for purposes of life-detection. (small, slow heat-output).

Different principles of calorimetry will be applied in new developments for the measurement of small total heat of instantaneous reactions The principal applications of this kind of calorimetry will presumably take place in the field of immunochemistry For those immuno-reactions which do not result in precipitation there is an urgent need for a novel analytical approach. Calorimetry as a method of chemical analysis is not, like all other analytical methods, limited to certain reactions or groups of reactions. It is applicable to every chemical change with the rarest exceptions. It is available where there is no other measurable sign of a suspected reaction. It demonstrates the absence as well as the presence of chemical change.

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(B) Chemosynthesis-project. Principal Investigator: Lutz Kiesow. The report-period, 1965, was the third active year of the Chemosynthesis-project. The two principal aims of the project were to discover two biochemical reaction-sequences:

(1) The sequence in which energy from inorganic sources is assimilated into the living world.

(2) The sequence in which carbon from the inorganic source, CO_2, is incorporated into living organisms.

Toward these two separate but closely related goals experimental efforts were directed with alternating emphasis since the inception of the project. Nevertheless, the energy-aspect was first of the two to be resolved.

Two years after completion of the needed facilities at the Bio-Energetics Laboratories, the mechanism of autotrophic energy assimilation was clarified and described in PNAS 52:980, (1964), "On the Assimilation of Energy From Inorganic Sources in Autotrophic Forms of Life". These findings have now been confirmed from other laboratories and by work of other authors with independent methods.

Our principal findings had been:

(1) The Reduction of Pyridine-Nucleotide, (as published from this laboratory in 1962) coupled with and driven by, the oxidation

of inorganic reducing agents which acts as the source of energy. This finding has been confirmed, not only with Nitrobacter (using NO_2^- as substrate) but also with Ferrobacteria (using Fe^{++} as substrate) by M. H. Aleem, Lees and Nicholas (Nature, London, 1963).

(2) The Thermodynamic Reversibility of the coupled oxido-reduction in which substrate (energy-donor) is oxidized while DPN is reduced to DPN.H (the product of energy-assimilation). This finding has been confirmed by P. Straat and A. Nason (J.B.C. 240:1412 (1965). These authors have also confirmed our findings of important characteristics of the enzyme-system involved, e.g. enzymic components, pH-optima and sensitivity to inhibition by KCN and other agents.

(3) The Disintegration of Water into protons and electrons as the initial step of energy assimilation. This observation, made by us with aneorobic nitrite oxidation (chlorate-experiment) has been confirmed by M. H. Aleem (PNAS 54:869 (1965). He repeated in a different way, - (with ^{18}O water) our finding that the oxygen in the nitrate of Nitrobacter derives from H_2O, not from molecular O_2.

Our findings thus confirmed in an independent manner rule out the explanations of photosynthesis given in the theories of Van Niel and Warburg. A third theory, that ATP is the product

of energy-assimilation, is no longer needed: With DPN.H identified as the product of energy-assimilation the subsequent production of ATP is explained as a result of the well-known process of cell-respiration (oxidative phosphorylation of ADP coupled with DPN.H-oxidation).

The experimental description of the assimilation of energy as described was thoroughly discussed and met with unreserved acceptance at the annual meeting of the Union of European Biochemical Societies in Berlin, Germany, where it was presented as the annual lecture of this year, on October 8.

The third year of research at Bethesda could now be devoted entirely to the problem of carbon assimilation. Progress has been achieved during the report period with the following steps:

(1) The energy-donor, DPN.H, was found to be an indispensable requirement for the assimilation of carbon in particles isolated from Nitrobacter.

(2) The energy-donor ATP was found to be an indispensable requirement for carbon-assimilation in Nitrobacter-particles.

(3) The acceptor-substance which incorporates the carbon atom of $H^{14}CO_3^-$ was isolated.

(4) The acceptor-substance was identified.

(5) The enzyme system for the combined interaction of HCO_3^-, the acceptor, DPN.H and ATP was found to be soluble.

(6) The intermediary compound formed by interaction of the acceptor with ATP was isolated.

(7) The intermediary compound was found to interact with DPN.H and HCO_3^-.

(8) The product of this interaction was isolated and its net-composition established. It is highly reactive metabolite.

(9) A specific enzyme-inhibitor was found which blocks the second, not the first of the two crucial reactions of the acceptor with ATP and DPN.H.

(10) It became possible to isolate substantial quantities of the first intermediary compound.

Findings (1) through (10) are essential steps toward a final clarification of the assimilation of carbon. It seems noteworthy that Nitrobacter also contains, in very small activities, and with minute reaction-rates, the elements of the Calvin-cycle: carbonydismutase, the carboxylating enzyme and the product of Calvin's carboxylation, phospho glyceric acid.

II. HUMAN ENERGETICS

Efforts at re-activating the human calorimeter facility with the accessories as used since 1947 (when the control circuits were designed by the American Society of Heating and Ventilating Engineers under contract with the Navy) have not succeeded. Whereas the gradient calorimeter as designed by the Bio-Energetics Laboratories of NMRI has maintained its reliable calibration now for 18 years, the accessory circuits, which supply liquid of controlled temperatures to the various units of the gradient calorimeter, have deteriorated beyond repair. In the state of the art of 1947 it was difficult to meet the specifications set by NMRI for controlling temperature in gradient layer devices and the accessory equipment was required to perfrom close to the limits of its performance. Nevertheless, it functioned during the entire period of construction, testing, and experimental application with flawless characteristics documented in calibrations after completion of the work.
In recent months it became difficult to obtain stability of temperature in the accessory liquid-supply circuits. A major job of reconstruction will have to be done, if the investment of the Navy in human calorimetry is to be protected and the tradition preserved in this field.
On May 21, 1965 Dr. L. Fox of OART, NASA, was informed of these difficulties. It was submitted that no application for funds would be forwarded to OART for the year of 1966 under Contract #R 8.

(Instead, the efforts of the Bio-Energetics Laboratories in the field of Molecular Energetics were intensified and an additional request for $25 000 over the previous annual grant of $50,000 for Molecular Energetics, was submitted and approved. These funds are intended for the development of microcalorimetric methods for detection of extraterrestrial life).

It seems appropriate, therefore to submit at this time a summary of progress in Human Energetics under #R-8 and # R-38, 1961 - 1965. This is done with two enclosures:

(1) A brief summary of research at the Bio-Energetics Laboratories on Human Thermoregulation and its relations to space-technology and

(2) A manuscript entitled: "The Thermal Homeostasis of Man" in press with Librairie Masson for the book commemorating Claude Bernard (Colloquium Claude Bernard, Paris, 1965).